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Research report

H_1-histamine receptors in the amygdala are involved in emotional memory but do not mediate anxiety-related behaviors in mice submitted to EPM testing

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ABSTRACT

This study investigated the role of amygdala H_1 receptors in state-dependent memory deficits induced by L-histidine (LH). Tests using an elevated plus-maze (EPM) were performed on two consecutive days: Trial 1 (T1) and Trial 2 (T2). Before each trial, mice were intraperitoneally (IP) injected with LH (500 mg/kg). Two hours later, they were microinjected with the H_1 receptor antagonist, chlorpheniramine (CPA 0.016, 0.052, or 0.16 nmol/0.1 μl), or saline (SAL) into the amygdala and submitted to the EPM. LH-CPA did not affect trial 1 performances in the EPM, which indicated that these drugs did not affect anxiety. Emotional memory, as revealed by a reduction in open arm exploration between both trials, was present in the SAL–SAL groups as well as in the SAL–CPA groups for the lower doses of CPA (0.016 and 0.052 nmol). On the contrary, neither the LH–SAL group nor the LH–CPA groups exhibited this decrease in open arm activity between both trials, which reveals that LH impaired emotional memory. While intra-amygdalar CPA did not interact with LH effect, it impaired per se the emotional memory performances at the highest dose (0.16 nmol). No significant changes were observed in the number of enclosed arm entries (EAE), an EPM index of general exploratory activity. These results may be attributed to a combined effect in the different nucleus of the amygdala. Taken together, these results suggest that the H_1 receptors in the amygdala are not implicated in anxiety-like behaviors but are involved in emotional states induced by the T1/T2 EPM protocol in mice.

1. Introduction

Neuronal histamine has been implicated in cognition functions, including learning and memory (Brown et al., 2001; Haas et al., 2008). Histamine functions are mediated through different receptors subtypes: H_1, H_2, H_3 and H_4 (Leurs et al., 1995; Strakhova et al., 2009). Additionally, high density of postsynaptically located histamine H_1 has been found in limbic regions, including the amygdala (Ryu et al., 1995). The amygdala has been known to play an important regulatory role in the acquisition of emotionally based learning and memory (Alvarez and Ruarte, 2002; Ribeiro et al., 2011); further, it is demonstrated a lateralized participation of the amygdala on exploratory behavior and histamine neurons appear mediate these differential modulations (Alvarez and Banzan, 2011). Anatomic data have shown that the amygdala receives afferences from the tuberomamillary nucleus (TM) of the posterior hypothalamus, the major source of brain histamine (Haas et al., 2008). Evidence has demonstrated that histamine can facilitate long-term potentiation (LTP) by activating histamine receptor subtypes (Haas et al., 2008; Luo and Leung, 2010). A study conducted by Jiang (2005) showed that *in vitro*, histamine can increase excitatory synaptic transmission in the amygdala; Furthermore, it is accepted that synaptic plasticity within the amygdala is the cellular basis of emotional memory because LTP is correlated with memory trace formation (Haas et al., 2008).

The widespread extension of histaminergic neurons modulating neuronal activity in other brain structures, such as the locus coeruleus that presents a pathway for neuromodulator interaction with the amygdala (Alvarez, 2009; Haas et al., 2008; McGaugh, 2004); it has been suggested that the histaminergic system may exert tonic modulatory control over emotional behavior (Santos

Abbreviations: LH, L-histidine; EPM, elevated plus-maze; T1, Trial 1; T2, Trial 2; intraperitoneally, IP; CPA, chlorpheniramine; SAL, Saline; %OAE, percentage of open arm entries; %OAT, percentage of open arm time; ANOVA, analysis of variance; OAE, open arm entries; EAE, enclosed arm entries; OAT, open arm time; EAT, enclosed arm time; CT, central area time; mPFC, Medial portion of the prefrontal cortex; 5-HT, Serotonin; I.C.V, intracerebroventricular; IA, Intra-amygdala; NBM, nucleus basalis magnocellularis.

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et al., 2001). In addition, some evidence supports the concept that histaminergic neurons influence learning and memory via H_1 histaminergic receptor activation (Alvarez and Banzan, 2008; Masuoka et al., 2010). Indeed, intra-hippocampal administration of histamine in rats has been shown to interfere with the consolidation of the avoiding response, affecting latency and memory efficiency, and this interference was mediated by histamine H_1 receptors (Alvarez and Banzan, 2008). In mice, intracerebroventricular administration of histamine H_1 receptor antagonist prevented the improving effect of pre-test lithium on memory retrieval (Zarrindast et al., 2008). In humans, oral administration of diphenhydramine, an H_1-receptor antagonist, induced attentional and working-memory impairments (Kay and Harris, 1999). Although the studies have indicated that histamine H_1 receptors could have a modulatory action on memory processes, few studies have investigated histamine effects mediated by H_1 receptors present in the amygdala on emotional memory using repeated testing in an elevated plus-maze test.

A model used to detect the influence of emotional states on memory in a repeated measures protocol is the elevated plus-maze (EPM) (Carobrez and Bertoglio, 2005). The EPM is a widely used test of animal anxiety (Lister, 1987; Pellow et al., 1985), and according to Galvis-Alonso et al. (2010), animals acquire information about safe and dangerous areas of the maze during EPM testing. Repeated testing provides a measure of acquisition and memory retention because experience-dependent behavioral changes can be observed. Recently, we demonstrated that intraperitoneal (IP) injection of L-histidine (LH), a precursor of histamine, induces a state-dependent memory retrieval deficit in mice when repeatedly exposed to the EPM (Gianlorenço et al., 2011; Serafim et al., 2010). However, our research group did not account for the involvement of H_1 receptor modulation in the amygdala on emotional behaviors.

Therefore, the aim of this study was to verify whether the effects of LH on emotional memory retrieval in mice are mediated by H_1 receptors in the amygdala. To address this question, we investigated the effects of microinjection H_1 antagonist chlorpheniramine (CPA) into the amygdala on anxiety and emotional memory in mice previously treated with LH and re-exposed to the EPM.

2. Material and methods

2.1. Animals

The experimental subjects were 117 adult male Swiss mice (Federal University of São Carlos, SP, Brazil) weighing 25–32 g at testing. The mice were housed in groups of 4 per cage (28 cm × 18 cm × 11 cm) and maintained under a 12-h light cycle (lights on at 7 A.M.) in a controlled environment at a temperature of 23 ± 1 °C and with a relative humidity of 50 ± 5%. Food and drinking water were provided *ad libitum*, except during the brief testing periods. All mice were experimentally naïve at the beginning of the study. The experimental sessions were conducted during the light period of the cycle (9 A.M.–4 P.M.).

2.2. Drugs

L-histidine hydrochloride (a precursor of histamine) (Sigma, MO, USA) and chlorpheniramine maleate salt (an H_1 receptor antagonist; Sigma Chemical Co., St. Louis, MO) were dissolved in sterile 0.9% saline solution. The LH solution was administered intraperitoneally (IP) at a volume of 2 ml/kg body weight; the final dose was 500 mg/kg. The CPA solution was microinjected at doses of 0.016 nmol, 0.052 nmol and 0.16 nmol in a volume of 0.1 μl. The doses used were based on previous studies (Kumar et al., 2007; Privou et al., 1998; Serafim et al., 2010) and on pilot work in our laboratory.

2.3. Surgery and microinjection

Mice were bilaterally implanted with a 7-mm stainless-steel guide cannula (25-gauge; Insight Equipamentos Científicos Ltda.) under general anesthesia with ketamine hydrochloride (100 mg/kg, IP) and xylazine (10 mg/kg, IP). Stereotaxic coordinates (Paxinos and Franklin, 2001) for the target site in the amygdala were measured in relation to the skull surface: antero-posterior (AP) = −0.8 mm, lateral (L) = ±2.7 mm and ventral (V) = −2.0 mm. The guide cannula was fixed to the skull using dental acrylic and jeweler's screws. During implantation, the guide cannula remained 1 mm above the target site. A dummy cannula (33-gauge stainless steel wire; Fishtex Industry and Commerce of Plastics Ltda.) was inserted into each guide cannula immediately after surgery to reduce the incidence of occlusion. Post-operative analgesia was provided for two days by adding acetaminophen (200 mg/ml) to the drinking water in a ratio of 0.2 ml acetaminophen to 250 ml water (i.e., a final concentration of 0.16 mg/ml) (Messier et al., 1999).

Three days after surgical recovery, solutions were injected into the amygdala using microinjection units (33-gauge stainless steel cannula; Insight Equipamentos Científicos Ltda.), which extended 1.0 mm beyond the tips of the guide cannulae. Each microinjection unit was attached to a 5-μl Hamilton microsyringe via polyethylene tubing (PE-10), and administration was controlled by an infusion pump (BI 2000; Insight Equipamentos Científicos Ltda.) programmed to deliver a volume of 0.1 μl (volume injected) over a period of 60 s. The microinjection procedure consisted of gently restraining the animal, removing the dummy cannula, inserting the injection needle, infusing the solution, and keeping the injection unit *in situ* for 60 s. Successful infusion was confirmed by monitoring the movement of a small air bubble in the PE-10 tubing.

2.4. Apparatus

The apparatus used for EPM testing was similar to those developed by Lister (1987), with a floor and walls constructed from acrylic. The maze was elevated to a height of 38.5 cm from the floor and consisted of two open (30 cm × 6 cm × 0.6 cm) arms opposite to two enclosed (30 cm × 6 cm × 15.5 cm) arms, extending from a common central platform (6 cm × 6 cm). All testing was conducted under moderate illumination (77 lux; measured on the central platform of the EPM) during the light phase of the diurnal cycle.

2.5. Experimental procedure

Three days after surgery, the animals were transported to the experimental room and left undisturbed for at least 1 h before testing to facilitate adaptation. Trial 1 (T1) and Trial 2 (T2) were performed on two consecutive days. In T1, mice received an IP injection of LH. Two hours later, they received an intra-amygdala microinjection of SAL or CPA. Both drugs were administered before each trial. In T2 (24 h later), the mice were again submitted to the pharmacological treatments under the same experimental conditions. Each test session began by placing the subject on the central platform of the maze, facing an open arm. The subject was allowed 5 min of free exploration. Between animals, the maze was thoroughly cleaned with 20% alcohol and dry cloths. All sessions were video recorded using a camera positioned above the maze and linked to a computer in an adjacent room. Injection procedure was done in the amygdaloid complex as described by Baptista et al. (2009) and Barbalho et al. (2009). Mice received an IP injection of LH, followed by an intra-amygdala infusion of SAL or CPA (0.016, 0.052, or 0.16 nmol/0.1 μl) 2 h later (Huang et al., 2003). In each trial, the

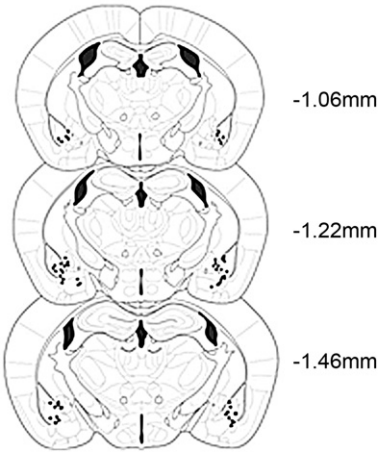

Fig. 1. Schematic representation of amygdala microinfusion sites in mice. Sections are between −1.06 and −1.46 mm from the bregma in the atlas of Paxinos and Franklin (2001). There are fewer sites than total mice because of overlaps.

drugs were administered prior to the test, and 5 min after the CPA infusion, each mouse was submitted to EPM testing. For each CPA dose administered, the animals were randomly assigned to four groups based on drug treatment: SAL–SAL (IP injection and intra-amygdala infusion of SAL), LH–SAL (IP injection of LH and infusion of SAL), SAL–CPA (IP injection of SAL and infusion of CPA) and LH–CPA (IP injection of LH and infusion of CPA).

2.6. Behavioral analysis

Videotapes were scored by a highly trained observer using an ethological analysis software package X-Plo-Rat developed at the Laboratory of Exploratory Behavior, USP, Ribeirao Preto (Becerra-García et al., 2005). Behavioral parameters were defined according to previous studies (Lister, 1987; Rodgers and Johnson, 1995) and included the following: frequencies of open and enclosed arm entries (OAE and EAE) (arm entry = all four paws into an arm), total time spent in open arms (OAT), total time spent in enclosed arms (EAT), and total time spent in the central area (CT). These data were used to calculate the percentages of OAE [%OAE; (OAE/TE) × 100] and OAT [%OAT; (OAT/300) × 100].

2.7. Histology

At the end of the experiments, each animal received a 0.1 μl infusion of 1% methylene blue according to the previously described microinjection procedure. The animals received an anesthetic overdose, their brains were removed and the injection sites were microscopically (Olympus B202) verified according to the atlas of Paxinos and Franklin (2001). Data from animals with injection sites outside the amygdala were excluded from analysis. Histological analysis confirmed that 117 mice had accurate cannula placement in the amygdala (Fig. 1) and that the sample sizes of the different dosage cohorts were as follows: [CPA 0.016 nmol/0.1 μl: SAL–SAL ($n = 11$), LH–SAL ($n = 11$), SAL–CPA ($n = 10$), LH–CPA ($n = 8$); CPA 0.052 nmol/0.1 μl: SAL–SAL ($n = 11$), LH–SAL ($n = 11$), SAL–CPA ($n = 9$), LH–CPA ($n = 8$); CPA 0.16 nmol/0.1 μl: SAL–SAL ($n = 10$), LH–SAL ($n = 11$), SAL–CPA ($n = 9$), LH–CPA ($n = 8$)].

As shown in Fig. 1, microinjection tips were positioned in the amygdala and the methylene blue was within the amygdaloid complex. Therefore, the present results can be interpreted as chemical stimulation of the amygdala.

2.8. Statistics

Initially, all results were submitted to Levene's test for homogeneity of variance. When appropriate, the data were square-root transformed and then analyzed using two-way repeated measures ANOVA (factor 1: treatment; factor 2: trial). Significant F tests were followed by post hoc Fisher's LSD tests (protected t-tests). In all cases, p values lower than 0.05 were required for significance.

2.9. Ethics

All procedures were approved by the Ethics Committee on Animal Experimentation of the Federal University of São Carlos (#028/2007) and were compliant with the recommendations of the Brazilian Society of Neuroscience and Behavior (SBNeC), which are based on the US National Institutes of Health Guide for the Care and Use of Laboratory Animals.

3. Results

3.1. Effects of IP injection of LH (500 mg/kg) and intra-amygdala infusion of SAL or CPA (0.016 nmol/0.1 μl) in mice exposed and re-exposed to the EPM

Fig. 2(A) and (B) shows the treatment effects of IP LH injection (500 mg/kg) and intra-amygdala infusion of SAL or CPA (0.016 nmol/0.1 μl) on EPM behavioral measures. Two-way ANOVA revealed no significant treatment effects for %OAE [$F_{(3,36)} = 2.44$, $p > 0.05$] and %OAT [$F_{(3,36)} = 1.30$, $p > 0.05$] or OAE and OAT ($p > 0.05$). Post hoc test did not show significant differences between SAL–SAL and SAL–CPA or LH–SAL groups in T1 (Table 1) for these measures, indicating that the drugs did not induce changes in anxiety level. Furthermore, ANOVA followed by Fisher's LSD test showed no significant difference between groups in T1 for EAT, CT, and EAE ($p > 0.05$ for all) during T1 (Table 1).

For T2, the ANOVA confirmed a statistically significant effect of trial on %OAE [$F_{(1,36)} = 12.17$, $p < 0.05$] and %OAT [$F_{(1,36)} = 34.38$, $p < 0.05$] and a statistically significant treatment × trial interaction for OAE [$F_{(1,36)} = 5.31$, $p < 0.05$] and %OAT [$F_{(1,36)} = 7.81$, $p < 0.05$]. Fisher's LSD test showed decreased open arm exploration (%OAE and %OAT) for the SAL–SAL and SAL–CPA groups (Fig. 2A and B). Post hoc tests also indicated a significant decrease in OAE and OAT ($p < 0.05$) for the SAL–SAL and SAL–CPA groups from T1 to T2, while the LH–SAL group only exhibited decreased OAE across trials (Table 1). Importantly, %OAE and %OAT did not decrease ($p > 0.05$) for the LH groups (LH–SAL or LH–CPA) in T2 (Fig. 2A and B). Furthermore, as indicated by ANOVA followed by post hoc comparisons, the T2 EAT increased [$F_{(1,36)} = 20.09$, $p < 0.05$] for the SAL–SAL and LH–SAL groups, but EAE did not differ significantly between trials for any experimental group ($p > 0.05$). Fisher's LSD test also indicated that CT decreased [$F_{(1,36)} = 12.14$, $p < 0.05$] for the SAL–SAL and LH–SAL groups (Table 1).

3.2. Effects of IP injection of LH (500 mg/kg) and intra-amygdala infusion of SAL or CPA (0.052 nmol/0.1 μl) in mice exposed and re-exposed to the EPM.

Fig. 3(A) and (B) show the treatment effects of IP LH injection (500 mg/kg) and intra-amygdala infusion of SAL or CPA (0.052 nmol/0.1 μl) on EPM behavioral measures. The ANOVA revealed no significant treatment effects on anxiety as represented by %OAE [$F_{(3,35)} = 0.27$, $p > 0.05$] and %OAT [$F_{(3,35)} = 2.38$, $p > 0.05$] or OAE and OAT ($p > 0.05$) in T1 (Table 1), indicating that LH or CPA had no effect on anxiety. Additionally, ANOVA followed by Fisher's LSD test indicated no statistically significant difference between groups in T1 for any other measures (EAT, CT and EAE; $p > 0.05$) (Table 1).

Table 1
Effects of IP LH injection (500 mg/kg) and intra-amygdala infusion of CPA (0.016, 0.052, or 0.16 nmol/0.1 μl) prior to Trial 1 (T1) and prior to Trial 2 (T2) on behavioral measures in mice re-exposed to EPM testing.

Behaviors	SAL–SAL		LH–SAL		SAL–CPA		LH–CPA	
	T1	T2	T1	T2	T1	T2	T1	T2
CPA 0.016 nmol/0.1 μl								
EAE	4.4 ± 0.8	3.6 ± 0.8	4.8 ± 0.5	3.0 ± 0.7	3.9 ± 0.9	3.3 ± 0.7	4.8 ± 0.3	6.7 ± 1.8
OAE	3.2 ± 0.5	0.8 ± 0.1*	3.1 ± 0.4	1.7 ± 0.2*	3.2 ± 0.2	0.8 ± 0.3*	3.2 ± 0.2	3.3 ± 0.7
OAT	44.0 ± 6.6	4.5 ± 1.6*	49.5 ± 13.5	32.1 ± 7.5	33.0 ± 7.9	8.4 ± 4.2*	33.3 ± 9.2	43.0 ± 11.3
EAT	192.0 ± 17.4	268.7 ± 10.1*	175.2 ± 13.4	246.1 ± 13.4*	227.9 ± 10.5	250.3 ± 15.7	195.2 ± 19.4	216.1 ± 16.9
CT	63.9 ± 16.9	26.7 ± 9.6*	75.2 ± 13.1	21.7 ± 8.6*	39.06 ± 9.5	41.2 ± 13.5	71.4 ± 9.0	40.8 ± 7.5
CPA 0.052 nmol/0.1 μl								
EAE	3.6 ± 0.8	3.0 ± 0.5	4.8 ± 0.5	3.0 ± 0.7	5.5 ± 1.2	2.1 ± 0.8*	6.0 ± 1.6	2.8 ± 0.6*
OAE	2.8 ± 0.3	0.5 ± 0.2*	3.1 ± 0.4	1.7 ± 0.2*	3.3 ± 0.4	1.0 ± 0.4*	2.5 ± 0.7	1.3 ± 0.5
OAT	31.1 ± 5.8	2.9 ± 1.2*	49.5 ± 3.5	32.1 ± 7.5	33.0 ± 7.3	3.6 ± 1.4*	24.3 ± 8.3	26.5 ± 11.1
EAT	185.4 ± 24.2	274.4 ± 8.8*	175.2 ± 13.4	246.1 ± 13.4*	218.7 ± 23.1	285.6 ± 3.3	218.0 ± 3.1	236.1 ± 19.8
CT	83.4 ± 7.2	22.5 ± 8.5*	75.2 ± 3.1	21.7 ± 8.6*	47.8 ± 16.8	10.7 ± 2.3*	57.6 ± 16.5	37.3 ± 11.5
CPA 0.16 nmol/0.1 μl								
EAE	2.8 ± 0.5	2.6 ± 0.5	4.8 ± 0.5	3.0 ± 0.7	3.7 ± 0.6	3.4 ± 0.8	4.7 ± 0.7	3.0 ± 0.2
OAE	4.1 ± 0.8	1.2 ± 0.4*	3.1 ± 0.4	1.7 ± 0.2*	2.4 ± 0.3*	1.6 ± 0.3	2.0 ± 0.3	1.1 ± 0.1
OAT	49.1 ± 11.3	10.4 ± 5.1*	49.5 ± 3.5	32.1 ± 7.5	20.9 ± 4.1	38.2 ± 13.9	34.3 ± 11.8	30.5 ± 4.1
EAT	162.5 ± 30.3	251.3 ± 16.2	175.2 ± 13.4	246.1 ± 13.4*	199.1 ± 18.2	227.6 ± 16.9	164.8 ± 22.3	246.9 ± 4.1*
CT	88.3 ± 26.6	38.2 ± 12.3*	75.2 ± 3.1	21.7 ± 8.6*	79.8 ± 16.8	34.1 ± 14.1	100.7 ± 26.9	22.5 ± 3.3*

Data are reported as mean ± SEM. TE = total entries; EAE = enclosed arm entries; OAE = open arm entries; OAT = time spent in open arms; EAE = time spent in enclosed arms; CT = time spent in the central area; *$p < 0.05$ for T1 compared to T2 (ANOVA, followed by Fisher LSD test).

As indicated in Fig. 3(A) and (B), ANOVA confirmed effects for the trial factor in %OAE [$F_{(1,35)} = 6.37$, $p < 0.05$] and %OAT [$F_{(1,35)} = 7.48$, $p < 0.05$], as well as in OAE and OAT ($p < 0.05$), but no statistically significant treatment × trial interactions were observed for these measures ($p > 0.05$). The post hoc comparisons indicated decreases in %OAE, %OAT, OAE and OAT (Table 1) for the SAL–SAL and SAL–CPA groups, while the LH–SAL group only exhibited a decrease in OAE from T1 to T2. However, open arm activity (%OAE and %OAT) did not decrease in either LH group (LH–SAL or LH–CPA) between T1 and T2. ANOVA followed by the Fisher's LSD test revealed a significant increase in EAT [$F_{(1,35)} = 5.66$, $p < 0.05$] for the SAL–SAL and LH–SAL groups and a decrease in EAE [$F_{(1,35)} = 19.99$, $p < 0.05$] for the SAL–CPA and LH–CPA groups in T2; only the LH–CPA group did not exhibit a significant decrease in CT [$F_{(1,35)} = 15.02$, $p < 0,05$] (Table 1).

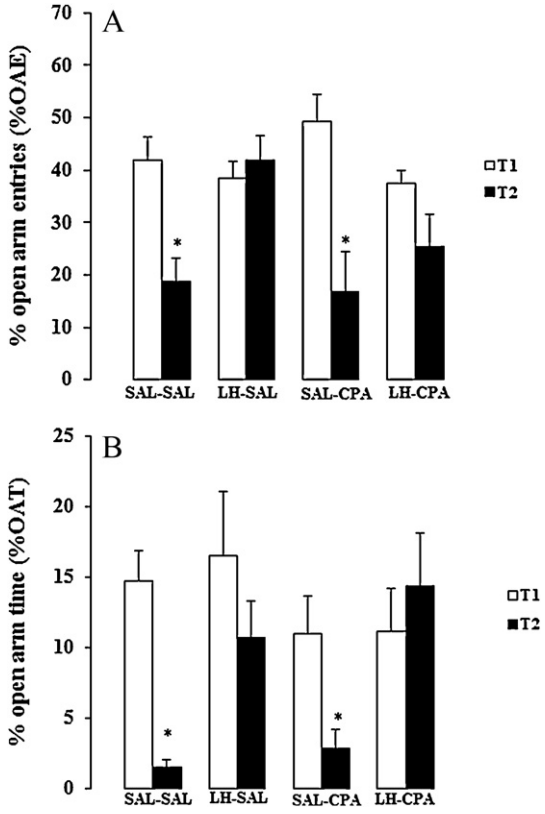

Fig. 2. Effects of IP LH injection (500 mg/kg) and intra-amygdala (IA) infusion of SAL or CPA (0.016 nmol/0.1 μl) on the percentage of open arm entries (%OAE) (A) and the percentage of time spent in open arms (%OAT) (B) during Trial 1 (T1) and Trial 2 (T2) in the EPM. Both drugs were administered prior to each trial. Groups: SAL–SAL (IP and IA injection of SAL), LH–SAL (IP injection of LH and IA SAL), SAL–CPA (IP injection of SAL and IA CPA), and LH–CPA (IP injection of LH and IA CPA). *$p < 0.05$ for T1 compared to T2 (ANOVA followed by Fisher's LSD test).

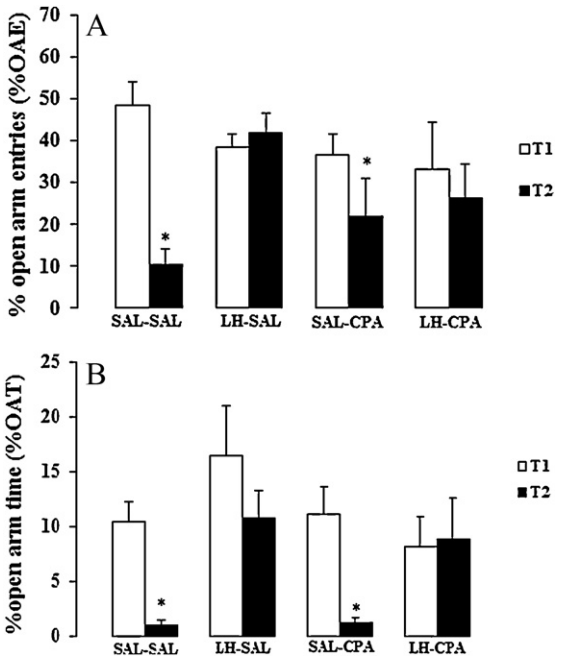

Fig. 3. Effects of IP LH injection (500 mg/kg) and intra-amygdala (IA) infusion of SAL or CPA (0.052 nmol/0.1 μl) on the percentage of open arm entries (%OAE) (A) and the percentage of time spent in open arms (%OAT) (B) during Trial 1 (T1) and Trial 2 (T2) in the EPM. Both drugs were administered prior to each trial. Groups: SAL–SAL (IP and IA injection of SAL), LH–SAL (IP injection of LH and IA SAL), SAL–CPA (IP injection of SAL and IA CPA), and LH–CPA (IP injection of LH and IA CPA). *$p < 0.05$ for T1 compared to T2 (ANOVA followed by Fisher's LSD test).

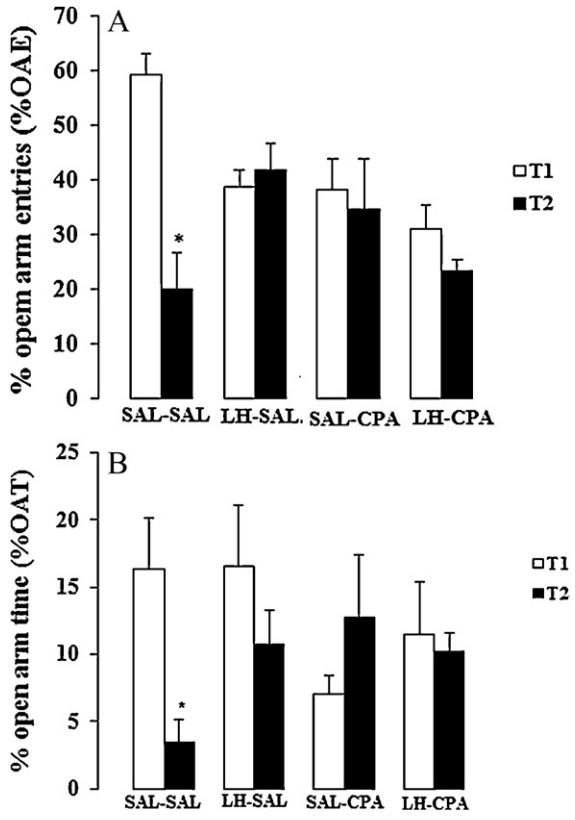

Fig. 4. Effects of IP LH injection (500 mg/kg) and intra-amygdala (IA) infusion of SAL or CPA (0.16 nmol/0.1 μl) on the percentage of open arm entries (%OAE) (A) and the percentage of time spent in open arms (%OAT) (B) during Trial 1 (T1) and Trial 2 (T2) in the EPM. Both drugs were administered prior to each trial. Groups: SAL–SAL (IP and IA injection of SAL), LH–SAL (IP injection of LH and IA SAL), SAL–CPA (IP injection of SAL and IA CPA), and LH–CPA (IP injection of LH and IA CPA). $^{*}p < 0.05$ for T1 compared to T2 (ANOVA followed by Fisher's LSD test).

3.3. Effects of IP injection of LH (500 mg/kg) and intra-amygdala infusion of SAL or CPA (0.16 nmol/0.1 μl) in mice exposed and re-exposed to the EPM

The treatment effects of IP injection of LH (500 mg/kg) and intra-amygdala infusion of SAL or CPA (0.16 nmol/0.1 μl) are shown in Fig. 4(A) and (B). The ANOVA revealed no statistically significant effects of treatment on the anxiety measures %OAE [$F_{(3,34)} = 1.87$, $p > 0.05$] and %OAT [$F_{(3,34)} = 0.40$, $p > 0.05$] or on OAE and OAT ($p > 0.05$) in T1 (Table 1), indicating that the drugs did not affect anxiety. As confirmed by two-way ANOVA followed by post hoc test, there were no significant difference between groups for EAT, CT and EAE ($p > 0.05$ for all) in T1 (Table 1).

ANOVA followed by Fisher's LSD test showed that the only statistically significant difference between exposures (trial effect) observed was for the SAL–SAL group, in which %OAE [$F_{(1,34)} = 8.51$, $p < 0.05$] and %OAT [$F_{(1,34)} = 2.17$, $p < 0.05$] decreased in T2; there was a treatment × trial interaction in %OAE [$F_{(1,34)} = 5.39$, $p < 0.05$]. The post hoc test indicated that the LH–SAL group exhibited decreased OAE during T2, but none of the other experimental groups (SAL–CPA and LH–CPA) significantly modified their open arm exploration in T2 ($p > 0.05$). Moreover, Table 1 shows that EAT increased significantly in T2 for the SAL–SAL, LH–SAL and LH–CPA groups [$F_{(1,34)} = 25.70$, $p < 0.05$], while CT [$F_{(1,34)} = 20{,}62$, $p < 0.05$] decreased for all groups except SAL–CPA. Post hoc comparisons also showed no changes in EAE in T2 compared to T1 ($p > 0.05$) for any experimental group (Table 1).

4. Discussion

The main results of this study demonstrate that systemic administration of L-histidine and intra-amygdala microinjection of chlorpheniramine (CPA) did not affect behavioral measures of anxiety. LH impairs emotional memory in a state dependent manner, in EPM procedure; CPA in the amygdala does not interact with LH effect, but induces impairment per se at the highest dose, in mice re-exposed to the elevated plus-maze. Importantly, no significant changes were observed in the number of enclosed arm entries (EAE) in T1, a parameter considered to be a valid measure of locomotor activity in the elevated plus-maze (Cruz et al., 1994). Since the amygdala is very small in mice and the drugs diffuse, these results may be attributed to a combined effect in the different nucleus of the amygdaloid complex.

Our results show that LH (500 mg/kg, IP) injected prior to T1 did not affect anxiety because there was no significant difference in open arm activity (%OAE and %OAT) between SAL–SAL and the LH–SAL group during T1. In contrast, Kumar et al. (2007) showed that IP LH injection before EPM exposure induced anxiogenic-like effects in mice. Other studies have also shown that histamine administered into the basolateral amygdala induces anxiogenic-like behaviors in rats exposed to the EPM (Bananej et al., 2012; Zarrindast et al., 2011). The discrepancies between our findings and these previously reported experiments could be related to experimental differences among the many factors that seem to influence aversion to the open arms, such as the time of day at which testing occurs (Griebel et al., 1993) and the levels of illumination in the testing room (García et al., 2011). However, our results are consistent with previous studies conducted in our laboratory, which have shown that IP LH (500 mg/kg) injection does not change anxiety levels in mice re-exposed to the EPM (Gianlorenço et al., 2011; Serafim et al., 2010). Intra-amygdala infusion of CPA (0.016, 0.052, or 0.16 nmol) also did not appear to affect anxiety because there was no significant difference between the SAL–SAL and the group that received a CPA infusion (SAL–CPA). Privou et al. (1998) reported that unilateral injection of CPA at a dosage of 20 μg or 0.1 μg into the nucleus basalis magnocellularis (NBM) provoked anxiolytic-like effects in rats. In a recent report, systemic injection and local injection of CPA into the medial portion of the prefrontal cortex (mPFC) attenuated anxiety and fearful responses in mice through the activation of serotoninergic system (5-HT) (Miyata et al., 2011). These discrepancies concerning the role played by H_1 receptors may be related to the different injection sites used to evaluate anxiety-like responses.

We detected a notable decrease in open arm activity (%OAE and %OAT) for the SAL–SAL and SAL–CPA groups at a dose of 0.016 or 0.052 nmol during T2. Our results indicate that learning occurred for these groups in T1 and that emotional memory was evoked in T2. However, our results showed that the LH–SAL groups that received IP LH injection did not exhibit decreased open arm activity during T2 relative to T1, indicating that these mice did not remember aversive information about the open arms during T2. Our results corroborate previous studies from our laboratory that showed that systemic LH injection induced retrieval deficits of aversive information of open arms in a state-dependent manner (Gianlorenço et al., 2011; Serafim et al., 2010). Our data are in accordance with other behavioral studies that have shown impairment in learning and memory induced by Histamine (Ahmadi et al., 2010; Rubio et al., 2001).

The anatomical organization of histaminergic neurons suggests that this system might influence the neuronal activity of large brain areas; one such area, the *locus coeruleus*, modulates the attentional state and presents a tight neuromodulatory interaction with the amygdala (McGaugh, 2004). In addition, some amines such as histamine have been shown to have a biphasic, non-linear dose

effect on memory processes, and these amines likely act on emotional arousal levels (Baldi and Bucherelli, 2005). In our view, the IP LH injection could shift the curve action of histamine and decreased activation in the *locus coeruleous.* It has been demonstrated that noradrenergic nucleus has a direct projection to the amygdala (McGaugh, 2004), suggesting that this pathway has an important role in modulating memory storage in rats (McIntyre et al., 2011) and during retrieval of emotional memories in humans (Sterpenich et al., 2006). Histamine effects would consequently decrease adrenergic activity in the amygdala, and thus, attention level and performance would also decrease.

Considering the evidence of the involvement of H_1 receptors in emotional learning and memory processes (Alvarez and Banzan, 2008; Zarrindast et al., 2002) and the well-described projections from the histaminergic nucleus to the amygdala, we hypothesized that the blockade of H_1 receptors in this structure would prevent LH effects on emotional memory retrieval. However, our results not only suggest that LH impairs emotional memory in EPM procedure; they further demonstrated that CPA in the amygdala does not interact with LH effect, but induces emotional memory impairment per se at the highest dose. It has been proposed that histaminergic system influences cognitive processes by modulating the cholinergic activation (Blandina et al., 2004). Histamine excites cholinergic neurons of the NBM through H_1 receptor activation (Khateb et al., 1995). Further, rats that received intrahippocampal administration of the histamine H_1 receptor antagonist pyrilamine increased the number of errors in the working memory task and these effects were significantly reduced by infusion of the histamine H_1 receptor agonist 2-pyridylethylamine (Nakazato et al., 2000). In other study, emotional reactivity to spatial novelty is altered in H1R knockout mice, possibly due to changes in amygdala acetylcholine levels (Zlomuzica et al., 2008). We suggest that the highest dose of H_1-antagonist chlorpheniramine could lead to decreased acetylcholinergic activity from the NBM to the amygdala, which would impair the emotional memory expression. Our results suggest that histamine exerts its procognitive effect on memory via an interaction with cholinergic system, but this hypothesis has not yet been tested.

Therefore, the present results may be due to the effect of histamine on one of the well-organized amygdalar projections, indicating that emotional behavior expression in rodents is under the tonic modulatory control of this neurotransmitter.

5. Conclusion

In conclusion, the results indicate that that L-histidine and H_1 receptors present in the amygdala are involved in emotional memory but do not mediate anxiety-related behaviors in mice submitted to EPM testing.

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